AMENDMENTS TO THE CLAIMS

1-8. (Canceled).

9. (Currently Amended) A method for real-time detecting and quantifying a first amplicon and a second amplicon in a PCR mixture comprising the steps of:

a) thermally cycling a PCR mixture, wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA intercalating dye, a first template and a second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first T_m and the second amplicon has a second T_m and the first T_m is less than the second T_m, wherein the first amplicon has a first melting curve and the second amplicon has a second melting curve and the first melting curve does not overlap with the second melting curve;

b) obtaining during each thermal cycle a first emission reading of the double stranded DNA intercalating dye at a first measuring temperature, wherein the first measuring temperature is between an annealing/extension temperature and the first T_m, and a second emission reading of the double stranded DNA intercalating dye at a second measuring temperature, wherein the second measuring temperature is between the first T_m and the second T_m;

c) quantifying the first amplicon comprising determining during each thermal cycle a first emission amount of the first amplicon, which is the difference between the first emission reading and the second emission reading, and using the first emission amount and corresponding thermal cycle number to obtain a first emission vs. cycle curve;

d) quantifying the second amplicon comprising determining during each thermal cycle a second emission amount of the second amplicon, which is the second emission reading, and using the second emission amount and

corresponding thermal cycle number to obtain a second emission vs. cycle curve; and

e) obtaining a standard emission vs. cycle curve and comparing the first emission vs. cycle curve with the standard emission vs. cycle curve to obtain the amount of the first amplicon and comparing the second emission vs. cycle curve with the standard emission vs. cycle curve to obtain the amount of the second amplicon.

10. (Canceled).

11. (Canceled).

12. (Previously Presented) The method of claim 9, wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

13. (Canceled).

14. (Canceled).

15. (Previously Presented) The method of claim 9, wherein the first measuring temperature is 0.25°C below the first T_m, 0.5°C below the first T_m, 1.0°C below the first T_m, 1.5°C below the first T_m, or 2.0°C below the first T_m, and wherein the first measuring temperature is higher than the annealing temperature.

16. (Previously Presented) The method of claim 9, wherein the second measuring temperature is 0.25°C below the second T_m, 0.5°C below the second T_m, 1.0°C below the second T_m, 1.5°C below the second T_m, or 2.0°C below the second T_m, and wherein the second measuring temperature is higher than the first T_m.

17. (Previously Presented) The method of claim 9, wherein the second measuring temperature is 0.25°C above the first Tm, 0.5°C above the first Tm, 1.0°C above the first Tm,

1.5°C above the first Tm, or 2.0°C above the first Tm, and wherein the second measuring temperature is less than the second Tm.

18. (Previously Presented) The method of claim 9, wherein the second measuring temperature is the first T_m + 0.25°C< the second measuring temperature < the second T_m-0.25°C, the first T_m + 0.5°C< the second measuring temperature < the second T_m-0.5°C, the first T_m + 1.0°C< the second measuring temperature < the second T_m-1.0°C, the first T_m + 1.5°C< the second measuring temperature < the second T_m-1.5°C, or the first T_m + 2.0°C< the second measuring temperature < the second T_m-2.0°C.

19. (Canceled).

20. (Previously Presented) The method of claim 9, wherein the first emission amount of the first amplicon is obtained through a computer program performing a calculation of subtracting the first emission reading from the second emission reading or subtracting the second emission reading from the first emission reading.

21. (Currently Amended) A method for real-time detecting and quantifying a first amplicon and a second amplicon in a PCR mixture comprising the steps of:

a) thermally cycling a PCR mixture, wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA intercalating dye, a first template and a second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first T_m and the second amplicon has a second T_m and the first T_m is less than the second T_m, <u>wherein the first amplicon has a first melting curve and the second amplicon has a second melting curve and the first melting curve does not overlap with the second melting curve</u>;

b) obtaining during each thermal cycle a first pre-T_m emission reading of the double stranded DNA intercalating dye at a measuring temperature, which is below the first T_m, and a first post-T_m emission reading of the double stranded DNA intercalating dye at a ~~at the~~ measuring temperature, which is

above the first T_m, and a second pre-T_m emission reading of the double stranded DNA intercalating dye at a measuring temperature, which is below the second T_m, and a second post-T_m emission reading of the double stranded DNA intercalating dye at the a measuring temperature which is above the second T_m;

c) quantifying the first amplicon comprising determining during each thermal cycle a first emission amount of the first amplicon, which is the difference between the first pre-T_m emission reading and the first post-T_m emission reading; and using the first emission amount and corresponding thermal cycle number to obtain a first emission vs. cycle curve;

d) quantifying the second amplicon comprising determining during each thermal cycle a second emission amount of the second amplicon, which is the difference between the second pre-T_m emission reading and the second post-T_m emission reading, and using the second emission amount and corresponding thermal cycle number to obtain a second emission vs. cycle curve; and

e) obtaining a standard emission vs. cycle curve and comparing the first emission vs. cycle curve with the standard emission vs. cycle curve to obtain the amount of the first amplicon and comparing the second emission vs. cycle curve with the standard emission vs. cycle curve to obtain the amount of the second amplicon.

22. (Canceled).

23. (Previously Presented) The method of claim 21, wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

24. (Canceled).

25. (Canceled).

26. (Previously Presented) The method of claim 21, wherein the measuring temperature below the first T_m and/or the second T_m are 0.25°C below, 0.5°C below, 1.0°C below, 1 .5°C below, or 2.0°C below.

27. (Previously Presented) The method of claim 21, wherein the measuring temperature above the first T_m and/or the second T_m are 0.25°C above, 0.5°C above, 1.0°C above, 1.5°C above, or 2.0°C above.

28. (Previously Presented) The method of claim 21, wherein the first emission amount of the first amplicon is obtained through a computer program performing the calculation of subtracting the first pre-T_m emission reading from the first post-T_m emission reading or subtracting the first post-T_m emission reading from the first pre-T_m emission reading, and the second emission amount of the second amplicon is obtained through the computer program performing the calculation of subtracting the second pre-T_m emission reading from the second post-T_m emission reading or subtracting the second post-T_m emission reading from the second pre-T_m emission reading.

29-84. (Canceled).

85. (Currently Amended) A method for real-time detecting and quantifying a first amplicon and a second amplicon in a PCR mixture comprising the steps of

a) thermally cycling a PCR mixture, wherein the PCR mixture comprises a thermostable polymerase, a double stranded DNA intercalating dye, a first template and a second template, primers for amplifying a first amplicon from the first template and a second amplicon from the second template, and wherein the first amplicon has a first T_m and the second amplicon has a second T_m and the first T_m is less than the second T_m, <u>wherein the first amplicon has a first melting curve and the second amplicon has a second melting curve and the first melting curve does not overlap with the second melting curve;</u>

b) obtaining during each thermal cycle a first emission reading of the double stranded DNA intercalating dye at a first measuring temperature, wherein the first measuring temperature is between an annealing/extension temperature and the first T_m, a second emission reading of the double stranded DNA intercalating dye at a second measuring temperature, wherein the second measuring temperature is between the first T_m and the second T_m, and a third emission reading of the double stranded DNA intercalating dye at a third measuring temperature, wherein the third measuring temperature is between the second T_m and a total denaturing temperature; and

c) quantifying the first amplicon comprising determining during each thermal cycle a first emission amount of the first amplicon, which is the difference between the first emission reading and the second emission reading, and using the first emission amount and corresponding thermal cycle number to obtain a first emission vs. cycle curve;

d) quantifying the second amplicon comprising determining during each thermal cycle a second emission amount of the second amplicon, which is the difference between the second emission reading and the third emission reading, and using the second emission amount and corresponding thermal cycle number to obtain a second emission vs. cycle curve; and

e) obtaining a standard emission vs. cycle curve and comparing the first emission vs. cycle curve with the standard emission vs. cycle curve to obtain the amount of the first amplicon and comparing the second emission vs. cycle curve with the standard emission vs. cycle curve to obtain the amount of the second amplicon.

86. (Canceled).

87. (Previously Presented) The method of claim 85, wherein the double stranded DNA intercalating dye is selected from the group consisting of ethidium bromide, YO-PRO-1, Hoechst 33258, SYBR Gold, and SYBR Green I.

88. (Canceled).

89. (Canceled).

90. (Previously Presented) The method of claim 85, wherein the first measuring temperature is 0.25°C below the first T_m, 0.5°C below the first T_m, 1.0°C below the first T_m, 1.5°C below the first T_m, or 2.0°C below the first T_m, and wherein the first measuring temperature is higher than the annealing temperature.

91. (Previously Presented) The method of claim 85, wherein the second measuring temperature is 0.25°C below the second T_m, 0.5°C below the second T_m, 1.0°C below the second T_m, 1.5°C below the second T_m, or 2.0°C below the second T_m, and wherein the second measuring temperature is higher than the first T_m.

92. (Previously Presented) The method of claim 85, wherein the second measuring temperature is 0.25°C above the first T_m, 0.5°C above the first T_m, 1.0°C above the first T_m, 1.5°C above the first T_m, or 2.0°C above the first T_m, and wherein the second measuring temperature is less than the second T_m.

93. (Previously Presented) The method of claim 85, wherein the second measuring temperature is the first T_m + 0.25°C < the second measuring temperature < the second T_m-0.25°C, the first T_m + 0.5°C < the second measuring temperature < the second T -0.5°C, the first T_m + 1.0°C < the second measuring temperature < the second T_m-1.0°C, the first T_m + 1.5°C < the second measuring temperature < the second T_m-1.5°C, or the first T_m + 2.0°C < the second measuring temperature < the second T_m-2.0°C.

94. (Previously Presented) The method of claim 85, wherein the third measuring temperature is 0.25°C above the second T_m, 0.5°C the second T_m, 1.0°C above the second T_m, 1.5°C above the second T_m, or 2.0°C above the second T_m, and wherein the third measuring temperature is less than the total denaturing temperature.

95. (Previously Presented) The method of claim 85, wherein the first emission amount of the first amplicon is obtained through a computer program performing a

calculation of subtracting the first emission reading from the second emission reading or subtracting the second emission reading from the first emission reading, and the second emission amount of the second amplicon is obtained through a computer program performing a calculation of subtracting the second emission reading from the third emission reading or subtracting the third emission reading from the second emission reading.

96. (Previously Presented) The method of claim 21, wherein the measuring temperature above the first T_m and the measuring temperature below the second T_m are the same.

97. (Previously Presented) The method of claim 9, wherein the first emission reading and the second emission are intermittently obtained during each thermal cycle.

98. (Previously Presented) The method of claim 21, wherein the first pre-T_m emission reading, the first post-T_m emission reading, the second pre-T_m emission reading, and the second post-T_m emission reading are intermittently obtained during each thermal cycle.

99. (Previously Presented) The method of claim 85, wherein the first emission reading, the second emission reading, and the third emission reading are intermittently obtained during each thermal cycle.

100-102. (Canceled).

103. (Currently Amended) The method of claim 9, wherein the first emission reading and the second emission reading are the only emission readings of the double stranded DNA intercalating dye ~~obtained~~ performed during each thermal cycle.

104. (Currently Amended) The method of claim 21, wherein the first pre-T_m emission reading, the first post-T_m emission reading, the second pre-T_m emission reading, and the second post-T_m emission reading are the only emission readings of the double stranded DNA intercalating dye ~~obtained~~ performed during each thermal cycle.

105. (Currently Amended) The method of claim 85, wherein the first emission reading, the second emission reading, and the third emission reading are the only emission readings of the double stranded DNA intercalating dye performed during each thermal cycle.

106. (Currently Amended) The method of claim 9, wherein the method consists of (a), (b), ~~and~~ (c), (d), and (e).

107. (Currently Amended) The method of claim 21, wherein the method consists of (a), (b), ~~and~~ (c), (d), and (e).

108. (Currently Amended) The method of claim 85, wherein the method consists of (a), (b), ~~and~~ (c), (d), and (e).

109. (Currently Amended) The method of claim 9, further comprising (i) ~~obtaining a standard emission vs. cycle plot, (ii)~~ obtaining a CT from the standard emission vs. cycle curve ~~plot~~ by positioning a fix emission line, ~~(iii) plotting~~ (ii) obtaining a curve of the log of an amount of DNA of a standard vs. a CT, wherein quantifying the first amplicon further comprises (a) ~~plotting the first emission amount obtained during each thermal cycle in an emission vs. cycle plot of the first amplicon, (b)~~ applying the fix emission line of ~~(ii)~~ (i) to obtain a CT of the first amplicon, ~~(c)~~ (b) using the CT of the first amplicon to obtain the log of the amount of DNA according to the curve ~~plot~~ of ~~(iii)~~ (ii), wherein quantifying the second amplicon further comprises (a) ~~plotting the second emission amount obtained during each thermal cycle in an emission vs. cycle plot of the second amplicon (b)~~ applying the fix emission line of ~~(ii)~~ (i) to obtain a CT of the second amplicon, ~~(c)~~ (b) using the CT of the second amplicon to obtain the log of the amount of DNA according to the curve ~~plot~~ of ~~(iii)~~ (ii).

110. (Currently Amended) The method of claim 21, further comprising (i) ~~obtaining a standard emission vs. cycle plot, (ii)~~ obtaining a CT from the standard emission vs. cycle curve ~~plot~~ by positioning a fix emission line, ~~(iii) plotting~~ (ii) obtaining a curve of the log of an amount of DNA of a standard vs. a CT, wherein quantifying the first amplicon further comprises (a) ~~plotting the first emission amount obtained during each thermal cycle in an emission vs. cycle plot of the first amplicon, (b)~~ applying the fix emission line of ~~(ii)~~ (i) to

obtain a CT of the first amplicon, ~~(c)~~ (b) using the CT of the first amplicon to obtain the log of the amount of DNA according to the curve ~~plot~~ of ~~(iii)~~ (ii), wherein quantifying the second amplicon further comprises (a) ~~plotting the second emission amount obtained during each thermal cycle in an emission vs. cycle plot of the second amplicon (b)~~ applying the fix emission line of ~~(ii)~~ (i) to obtain a CT of the second amplicon, ~~(c)~~ (b) using the CT of the second amplicon to obtain the log of the amount of DNA according to the curve ~~plot~~ of ~~(iii)~~ (ii).

111. (Currently Amended) The method of claim 85, further comprising (i) ~~obtaining a standard emission vs. cycle plot, (ii)~~ obtaining a CT from the standard emission vs. cycle curve ~~plot~~ by positioning a fix emission line, ~~(iii) plotting~~ (ii) obtaining a curve of the log of an amount of DNA of a standard vs. a CT, wherein quantifying the first amplicon further comprises (a) ~~plotting the first emission amount obtained during each thermal cycle in an emission vs. cycle plot of the first amplicon, (b)~~ applying the fix emission line of ~~(ii)~~ (i) to obtain a CT of the first amplicon, ~~(c)~~ (b) using the CT of the first amplicon to obtain the log of the amount of DNA according to the curve ~~plot~~ of ~~(iii)~~ (ii), wherein quantifying the second amplicon further comprises (a) ~~plotting the second emission amount obtained during each thermal cycle in an emission vs. cycle plot of the second amplicon (b)~~ applying the fix emission line of ~~(ii)~~ (i) to obtain a CT of the second amplicon, ~~(c)~~ (b) using the CT of the second amplicon to obtain the log of the amount of DNA according to the curve ~~plot~~ of ~~(iii)~~ (ii).

112. (Previously Presented) The method of claim 9, wherein each of the primers is not covalently linked to a dye.

113. (Previously Presented) The method of claim 21, wherein each of the primers is not covalently linked to a dye.

114. (Previously Presented) The method of claim 85, wherein each of the primers is not covalently linked to a dye.

115. (New) The method of claim 9, wherein the first amplicon is quantified using a computer program performing a calculation of the mathematical relationship between the first

emission amount and each thermal cycle number and the second amplicon is quantified using a computer program performing a calculation of the mathematical relationship between the second emission amount and each thermal cycle number.

116. (New) The method of claim 21, wherein the first amplicon is quantified using a computer program performing a calculation of the mathematical relationship between the first emission amount and each thermal cycle number and the second amplicon is quantified using a computer program performing a calculation of the mathematical relationship between the second emission amount and each thermal cycle number.

117. (New) The method of claim 85, wherein the first amplicon is quantified using a computer program performing a calculation of the mathematical relationship between the first emission amount and each thermal cycle number and the second amplicon is quantified using a computer program performing a calculation of the mathematical relationship between the second emission amount and each thermal cycle number.